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Kinetic modelling for flavanol extraction from red grape (*Vitis vinifera* L.) pomace using aqueous organic acid solutions

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Abstract

Grape pomace is a food industry waste containing a high burden of antioxidant polyphenols and several methodologies have been developed for their efficient extraction. However, a sustainable and environmentally friendly process should involve recovery means composed of benign, non-toxic solvents. In this line, this study examined the extraction of flavanols, a particular class of functional polyphenols occurring in grape pomace, using aqueous acetic acid and citric solutions. The aim was to assess the role of acid concentration in the extraction yield, employing kinetics. The kinetic model established enabled the reliable comparison of the extraction efficiency of acetic acid and citric acid solutions as a function of acid concentration. The results showed that acetic acid solutions are more suitable for retrieving flavanols from grape pomace. Using a 4% (w/v) acetic acid solution, a maximum total flavanol yield of 80 µg catechin equivalents per g of dry pomace could be achieved.

Introduction

Food industry companies are under legislative pressure to utilise waste materials in environmentally rational ways, to minimise associated risks (Devesa-Rey *et al.*, 2011). Wine production on an industrial scale generates large volume of wastes, mainly materials composed of organic substances (skins, seeds and stems). In Europe, it is estimated that 14.5 million tonnes of grape by-products are annually produced from the winemaking industry (Pinelo *et al.*, 2006). This residual material has been given particular attention, owed to the high richness in polyphenolic substances, which possess several properties, beneficial to human health (Yu and Ahmedna, 2013).

The investigations on the efficient retrieval of polyphenols from winery wastes have been focused on red pomace, which is characterised by relatively high content in polyphenols (Makris *et al.*, 2007), a large amount of which are flavanols. This particular class of flavonoids, represented principally by catechin, epicatechin and several dimers thereof, has been very extensively studied because of their unique antioxidant, antiatherogenic and anticarcinogenic properties (Rasmussen *et al.*, 2005). Thus the recovery of flavanols from grape industrial residues and their reuse as additives in foods and cosmetics, but also as dietary supplements, is one of the highest value options (Galanakis, 2012).

A plethora of approaches have been employed to develop methodologies for efficient extraction of polyphenols from wine industry residues. These approaches have focused on using benign and environmentally compatible means, such as hydroalcoholic solutions (Bucić-Kojić *et al.*, 2007; Makris *et al.*, 2008; Boussetta *et al.*, 2012; Carrera *et al.*, 2012). In studies pertaining to optimising the extraction process, emphasis has been given to the fact that pH is a parameter that may significantly affect extraction yield (Makris *et al.*, 2008), but also act as a factor for selective extraction, since flavanol extraction was shown to be favoured at pH lower than 3 (Karvela *et al.*, 2009a,b). Thus pH regulation by adding an acidifying agent in the extracting media used, might impact significantly the overall process efficiency.

Citric acid has been used in quite a few studies on polyphenol extraction from agri-food waste materials, such as onion peels (Kiassos *et al.*, 2009; Makris, 2010) and olive leaves (Mylonaki *et al.*, 2008). However, the effect of other natural, organic acids has never been investigated. Given that citric and acetic acid are natural, organic acids occurring widely in foods and can be produced through fermentation, it would appear that a more thorough examination on the use of these acids in extraction processes could be a further step into an integrated exploitation of waste materials from the food industry. In this framework, this study was undertaken in the view of assessing

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the effect of citric and acetic acid on the recovery of flavanols from red grape pomace. The extraction process was modelled with regard to extraction time and acid concentration, using kinetics.

Materials and Methods

Chemicals

p-Dimethylaminocinnamaldehyde (DMACA) reagent, and catechin acid were from Sigma Chemical Co (St. Louis, MO, U.S.A.). Acetic acid and citric acid were from Merck (Darmstad, Germany).

Grape pomace

Grape pomace originating from vinification of Agiorgitiko variety (*Vitis vinifera* spp.) was kindly provided by the Laboratory of Œnology (Department of Food Science & Human Nutrition, Agricultural University of Athens). The pomace was dried in an oven at 65°C for 48 h and then pulverized into a fine powder in a domestic blender. The pulverized material was kept at -20°C.

Extraction procedure

An aliquot of 2.4 g of pulverised grape pomace was added in 120 mL of either acetic or citric acid solution (1, 2 or 4% w/v), in a 250-mL glass vial and extractions were performed under stirring with a magnetic stirrer, at 80 rpm and 20°C. Sampling was carried out by removing 1 mL of extract at predetermined intervals over a period of 320 min.

Determination of the extraction yield in total flavanols (Y_{TF})

Samples were centrifuged in an Eppendorf table centrifugator at 10,000×g for 10 min prior to analysis. Determination of total flavanols was carried out employing the DMACA methodology (Makris *et al.*, 2008), using catechin as the calibration standard. Yield in total flavanols (Y_{TF}) was expressed as μg catechin equivalents (CTE) per g of dry pomace weight (dpw), by the following equation:

$$Y_{TF}\,(\mu g\ CTE\ g^{-1}\ dpw) = \frac{(15.15\times A_{640}+0.395)\times V}{m} \qquad (1)$$

Where V is the volume of the extraction medium (mL) and m the dry weight of grape pomace (g).

Statistical analysis

All determinations were carried out at least in triplicate and values were averaged and given along the standard deviation (± S. D.). All correlations were established using non-linear regression, at least at a 95% significance level ($p < 0.05$). For all statistics, SigmaPlot™ 12.0 and Microsoft Excel™ 2010 were used.

Results and Discussion

Kinetics

The model fitted to the extraction kinetics using non-linear regression between Y_{TF} values and t (Figure 1), was a hyperbola described by the equation:

$$y = \frac{ax}{1+bx} \qquad (2)$$

For all concentrations tested, fitting was statistically significant (Table 1), suggesting that extraction yield as a function of t can be adequately predicted by the eq. (2). This equation actually describes a 2nd-order extraction rate, as previously reported (Ho *et al.*, 2005; Rakotondramasy-Rabesiaka *et al.*, 2007; Pan *et al.*, 2012), when the boundary conditions t = 0 to t and $Y_{TF(t)} = 0$ to $Y_{TF(t)}$ are considered. The 2nd-order extraction rate might indicate that there are two phases implicated in the leaching of flavanols from the solid particles; first, there is a high rate of extraction, possibly owed to the most polar molecules; afterwards, the rate is significantly slowed down, suggesting extraction of less polar substances.

These considerations were made on the concession that (i) flavanols leached from the solid parts into the solution through diffusion, and (ii) at saturation conditions Y_{TF} remained constant. Based on such a simplification, the integrated law for the extraction kinetics would be:

$$Y_{TF(t)} = \frac{Y^{2}_{TF(s)}kt}{1+Y_{TF(s)}kt} \qquad (3)$$

Where $Y_{TF(s)}$ and k represent the TF yield at saturation and the extraction rate constant, respectively. Transformation of eq. (3) yields the following linearized form:

$$\frac{t}{Y_{TF(t)}} = \frac{1}{kY^{2}_{TF(s)}} + \frac{t}{Y_{TF(s)}} \qquad (4)$$

When t approaches 0, the initial extraction rate, h, given as $Y_{TF(t)/t}$, is defined as:

$$h = kY^{2}_{TF(s)} \qquad (5)$$

Plotting $t/Y_{TF(t)}$ versus t, would give a straight line in the form of y = ax + b (Figure 2), where a = $1/Y_{TF(s)}$ and b = 1/h. Thus for each temperature tested, $Y_{TF(s)}$, k and h could be determined graphically. In both cases the correlations between $t/Y_{TF(t)}$ and t were very high and statistically significant ($R^2 > 0.99$, $p < 0.0001$), which enabled the credible determination of the kinetic parameters that were descriptive of the

Table 1. Statistical parameters describing the correlation between Y_{TF} and t, established by using non-linear regression.

C_{acid} (% w/v)	Statistical parameters R^2	 *p*
Acetic acid		
1	0.98	<0.0001
2	1.00	<0.0001
4	0.99	<0.0001
Citric acid		
1	0.99	<0.0001
2	0.98	<0.0001
4	0.99	<0.0001

Table 2. Parameters of 2^{nd}-order kinetics, determined for the extraction of TF using acetic acid and citric acid solutions.

C_{acid} (% w/v)	Kinetic parameters k ($g\ \mu g^{-1}\ min^{-1}$) × 10^{-3}	 h ($\mu g\ g^{-1}\ min^{-1}$)	 $Y_{TF(s)}$ (μg CTE g^{-1} dpw)
Acetic acid			
1	6.98	14.57	45.7
2	2.60	10.77	64.4
4	1.53	9.84	80.0
Citric acid			
1	1.90	5.05	51.6
2	2.28	5.30	48.2
4	3.06	9.66	56.2

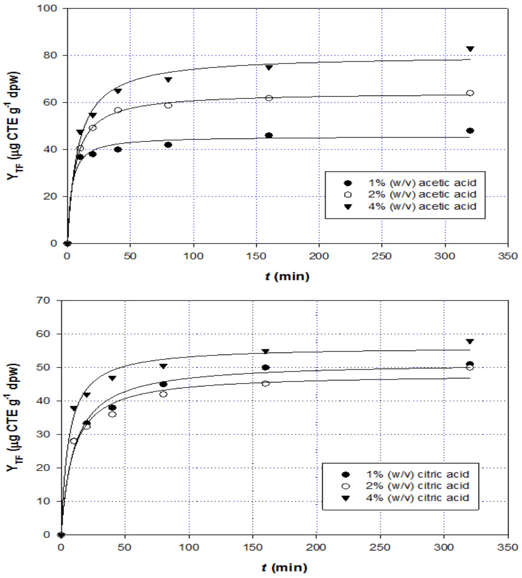

Figure 1. Non-linear regression between YTF and t values, during extraction of TF from grape pomace using acetic acid (upper plot) and citric acid (lower plot) solutions. Extractions were carried out at 20°C, 80 rpm and 1:50 solid-to-liquid ratio.

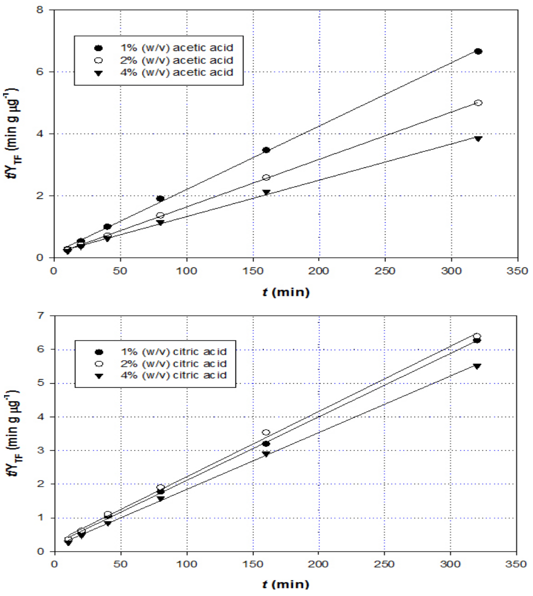

Figure 2. Second-order kinetics of TF extraction from grape pomace using aqueous acetic acid (upper plot) and citric acid (lower plot) solutions. Extractions were carried out at 20°C, 80 rpm and 1:50 solid-to-liquid ratio.

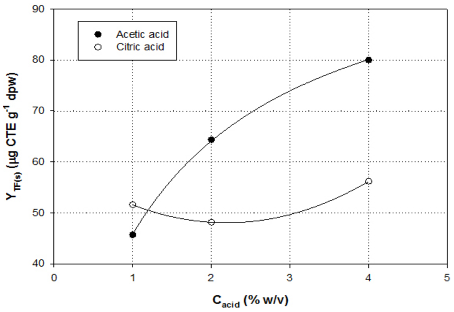

Figure 3. Non-linear regression plot displaying the correlation between $Y_{TF(s)}$ and C_{acid} values.

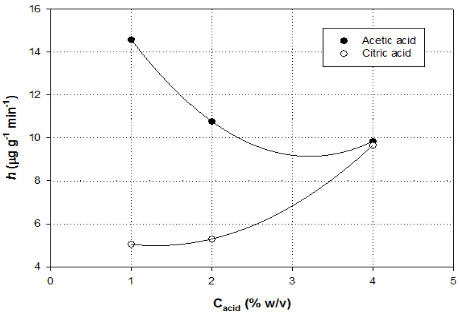

Figure 4. Non-linear regression plot displaying the correlation between h and C_{acid} values.

extraction process (Table 2).

Effect of acid concentration

As can be seen in Table 2, the highest values for both k and h were achieved using 1% (w/v) acetic acid solution. Increasing acetic acid concentration resulted in decreased k and h values. By contrast, $Y_{TF(s)}$ displayed an increasing tendency in response to increased acetic acid concentration and the highest $Y_{TF(s)}$ was attained with 4% (w/v) acetic acid. Irrespective of the t/C_{acid} combinations used, all three kinetic parameters determined were constantly lower in citric acid solutions. This finding is in agreement with early investigations, which showed that aqueous acetic acid solutions were more effective than citric acid ones, in extracting anthocyanins from grape pomace (Metivier *et al.*, 1980). Similar trends were reported for flavonoid recovery from onion solid wastes, using various combinations of acetic acid and citric acid with aqueous ethanol (Khiari *et al.*, 2008).

In the case of acetic acid solutions, non-linear regression between $Y_{TF(s)}$ and Cacid values (Figure 3) was shown to obey a single rectangular hyperbola

Table 3. Observed and predicted values of Y_{TF} for a number of runs performed to assess the validity of the extraction models established.

Run	Time (min)	C_{acid} (% w/v)	Y_{TF} (µg CTE g^{-1} dpw)			
			Acetic acid		*Citric acid*	
			Observed	Predicted	Observed	Predicted
1	10	1	36.8	34.9	28.1	38.1
2	160	1	46.0	45.0	50.0	50.5
3	320	1	48.0	45.4	51.0	51.0
4	10	2	40.5	40.2	28.0	33.3
5	160	2	61.9	61.8	45.2	46.8
6	320	2	64.0	62.9	50.0	47.5
7	10	4	47.6	44.2	38.0	35.8
8	160	4	75.1	76.2	55.0	54.2
9	320	4	83.0	78.1	58.0	55.2

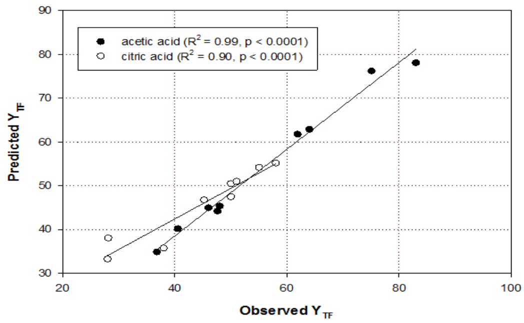

Figure 5. Correlation between observed Y_{TF} values and those determined using the predictive models established [eq. (11) and (12)].

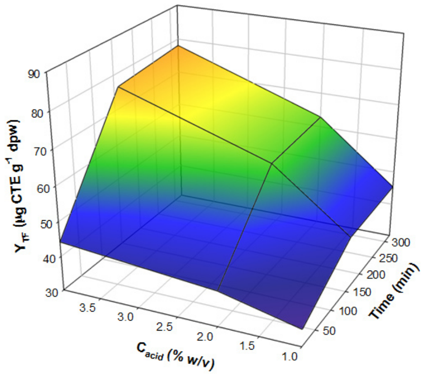

Figure 6. Three-dimensional plot showing the effect of C_{acid} and t in Y_{TF}, using acetic acid solutions.

correlation. This function was described by the following equation:

$$Y_{TF(s)} = \frac{106.62\times C_{acid}}{1.326+C_{acid}} \quad (R^2 = 0.999,\ p < 0.0001) \qquad (6)$$

In the same fashion, non-linear regression between h and C_{acid} values (Figure 4) was shown to obey a quadratic correlation, described by the following equation:

$$h = 1.112C_{acid}^2 - 7.135C_{acid} + 20.593 \quad (R^2 = 1.000,\ p < 0.0001) \qquad (7)$$

Likewise, the corresponding equations for citric acid solutions, obtained after implementing non-linear regression were:

$$Y_{TF(s)} = 2.482C_{acid}^2 - 10.875C_{acid} + 59.970 \quad (R^2 = 1.000,\ p < 0.0001) \qquad (8)$$

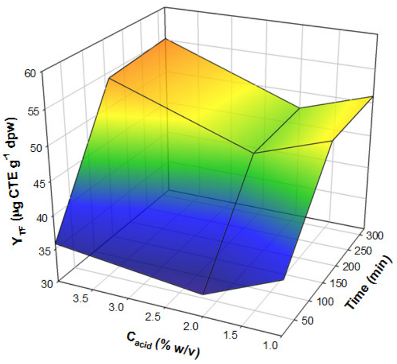

Figure 7. Three-dimensional plot showing the effect of C_{acid} and t in Y_{TF}, using citric acid solutions.

$$h = 0.643C_{acid}^2 - 1.68C_{acid} + 6.087 \quad (R^2 = 1.000,\ p < 0.0001) \qquad (9)$$

After rearrangement of eq. (3), Y_{TF} at any time, t, can be calculated:

$$Y_{TF(t)} = \frac{t}{\frac{1}{h}+\frac{t}{Y_{TF(s)}}} \qquad (10)$$

Thus for acetic acid solutions, combining eq. (6), (7) and (10), the following equation is obtained:

$$Y_{TF(t,C_{acid})} = \frac{t}{\frac{1}{1.112C_{acid}^2-7.135C_{acid}+20.593}+\frac{t}{\frac{106.62C_{acid}}{1.326+C_{acid}}}} \qquad (11)$$

In the same manner, combining eq. (8), (9) and (10), the corresponding expression for citric acid solutions would be:

$$Y_{TF(t,C_{acid})} = \frac{t}{\frac{1}{0.643C_{acid}^2-1.68C_{acid}+6.087}+\frac{t}{2.482C_{acid}^2-10.875C_{acid}+59.97}} \qquad (12)$$

These empirical equations represent the evolution model of Y_{TF} during extraction of flavanols from grape pomace with either aqueous acetic or citric acid and provide the values for Y_{TF} at any time t and any concentration C_{acid} ranging between 10 and 320 min, and 1 and 4% (w/v), respectively.

Experimental fitting (model validation)

A series of nine combinations of C_{acid} and t were used to test the validity of the model in predicting Y_{TF} values (Table 3). The observed and the predicted values were then analysed by linear regression to ascertain the degree of correlation (Figure 5). In both cases it was found that the observed and the predicted values were highly correlated, suggesting that, under the given experimental conditions, Y_{TF} can be calculated with high reliability as a function of Cacid and t, using the eq. (11) and (12). The tendencies in Y_{TF} recorded in each case were given in the form of three-dimensional plots (Figures 6 and 7).

Conclusions

Flavanol recovery from grape pomace using acetic acid and citric acid solutions was shown to be affected by both the type of the acid and its concentration. The course of the extraction was approached by the determination of basic kinetic parameters, which permitted the building of a predictive model. Using this model the yield of flavanol extraction, as a function of both t and C_{acid} could be very reliably estimated, under the experimental conditions deployed. This study showed for the first time that aqueous media used for extracting bioactive substances from residual plant materials can be profoundly affected by the acidifying agent and its concentration. This is particularly important in developing an efficient process, where maximum recovery is always sought. It is anticipated that similar studies might be of significant value in engineering extraction processes using environmentally benign and food compatible solvent systems. Currently, work is in progress to investigate the effect of other critical parameters affecting the extraction process, such as temperature.

Nomenclature

C_{acid}, acid concentration (% w/v)
CTE, catechin equivalents
DMACA, p-dimethylaminocinnamaldehyde
dpm, dry pomace weight
h, initial extraction rate (μg g^{-1} min^{-1})
k, extraction rate constant (min g $μg^{-1}$)
t, time (min)
TF, total flavanols
Y_{TF}, extraction yield in total flavanols (μg CTE g^{-1} dpw)
$Y_{TF(s)}$, extraction yield in total flavanols at saturation (μg CTE g^{-1} dpw)
$Y_{TF(Cacid,t)}$, extraction yield in total flavanols as a function of C_{acid} and t

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